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(54) **[Title of the Invention] A Beautifying and Whitening Cosmetic Material**

(57) [Abstract]

[Objective] To provide a beautifying and whitening cosmetic material which has a superior whitening effect on the skin and which has sufficient storage stability and high safety.

[Structure] First, approximately 100 g of dried mulberry cuttings, which is a crude drug, is pulverized in a mixer, the pulverized matter and 1 liter of 50% ethyl alcohol are introduced into a flask and refluxing and extraction are performed at 50°C as the materials are being stirred. After extraction, this solution is suctioned and filtered and the filtrate that is obtained is concentrated at 50°C using an evaporator. Next, the concentrated solution that is obtained is dried under reduced pressure and 9.2 g of brown crystals (extracted matter) is obtained. The extracted matter that is obtained is compounded as the effective component with 0.01 to 5.0% of cosmetic material.

[Claims]

[Claim 1] A beautifying and whitening cosmetic material characterized in that an extracted material of at least one crude drug is selected from a group comprised of mulberry cuttings, black soybeans, achene of Siberian cocklebur [Xanthium sibiricum] and Milletia reticulata.

[Detailed Description of the Invention]

[0001]

[Field of industrial use] This invention relates to a beautifying and whitening cosmetic material having a skin whitening effect. In further detail, it relates to a beautifying and whitening cosmetic material, a crude drug extract, having a whitening effect based on a melanin production inhibiting action as the effective component.

[0002]

[Prior art] It is generally thought that black color, spots and freckles due to sunburn are produced by production of melanin, which is a blackish-brown amorphous pigment. When the skin is subjected to the external irritation of ultraviolet rays, tyrosinase (a tyrosine oxidizing enzyme), which is present in the melanin cells of the skin, is activated and tyrosine, which is a structural amino acid of proteins, is oxidized, with melanin being produced. Consequently, a skin whitening effect can be anticipated by inhibiting the activity of tyrosinase, which is involved in melanin production. For this reason, compounding of a tyrosinase activity inhibiting component with a cosmetic material has been proposed.

[0003] Known beautifying and whitening cosmetic materials having a beautifying and whitening effect include substances obtained by compounding of ascorbic acid or derivatives thereof as disclosed in Japanese Patent Announcement 55-43443 [1980], "A Beautifying and Whitening Cosmetic Material," and in Japanese Patent Announcement 54-974 [1979], "A Crude Drug Extract Compounding Composition." Other known substances having beautifying and whitening effects include topical skin agents in which arbutin is compounded (Japanese Patent Application Early Disclosure No. 60-16906 [1985]), bleaching cosmetic materials in which kojic acid is compounded (Japanese Patent Announcement 32-8100 [1957])

and cosmetic materials extracted from plant components (Japanese Patent Application Early Disclosure No. 63-2913 [1988]) and animal components (Japanese Patent Application Early Disclosure No. 63-8312 [1988]).

[0004] However, many of the aforementioned conventional cosmetic materials do not have a sufficient beautifying and whitening effect. In addition, many of them do not have sufficient storage stability and exhibit safety problems with respect to the skin such as exhibiting irritating effects.

[0005]

[Problems the invention is intended to solve] This invention has the objective of solving the problems of the aforementioned conventional technologies and of providing a beautifying and whitening cosmetic material having a beautifying whitening effect and having sufficient storage stability and high safety.

[0006]

[Means for solving the problems] The inventors conducted intensive research for the purpose of solving these problems. As a result, we discovered that extracts of raw drugs such as mulberry cuttings, black soybeans, achene of Siberian cocklebur [Xanthium sibiricum] and Milletia reticulata have a tyrosinase enzyme activity inhibiting action and a melanin production inhibiting action in melanoma cells.

[0007] Specifically, this invention provides a beautifying whitening cosmetic material characterized in that an extracted material of at least one crude drug is selected from a group comprised of mulberry cuttings, black soybeans, achene of Siberian cocklebur [Xanthium sibiricum] and Milletia reticulata.

[0008]

[Action] The cosmetic material of this invention can be manufactured by the following method. First, a dried material or pulverized fried material consisting of at least one of the following, mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata is heated and extracted using an extraction solvent. Said extraction solvent can be an alcohol (methanol, ethanol, propanol or isopropanol) or water. In addition, mixed solutions of these substances can also be used. For example, when an aqueous solution of alcohol, with an alcohol concentration of 20 to 70% is used and extraction is performed for 1 hour at 50°C, there is excellent extraction.

[0009] After extraction, the extraction solution is separated by filtration and an extraction extract [sic] is obtained. The extraction extract is then concentrated and dried under reduced pressure as it is being heated at a temperature of under 60°C, the dried extract is recovered and is compounded with a cosmetic material. The aforementioned extraction extract may also be compounded with a cosmetic material in unaltered form.

[0010] The inventors confirmed that the extract of the crude drug (mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata) that has been obtained in this way exhibits a superior melanin production inhibiting action at lower concentrations than ascorbic acid, which is used conventionally. A whitening cosmetic material having a beautifying whitening effect can be obtained by compounding this extract as the effective component in amounts of 0.01 to 5.0%.

[0011] We shall now present a more detailed description of this invention by means of examples. However, the scope of this invention is not limited by the following examples.

[0012]

[Example 1] This example illustrates one of the methods of extraction of crude drug. First, approximately 100 g of dried mulberry cuttings, which was the crude drug, was pulverized with a mixer, the pulverized material and 500 ml of 50% ethyl alcohol were introduced into a flask, and refluxing and extraction were performed for 1 hour at 50°C as the materials were being stirred. After extraction, this solution was filtered by suction and the filtrate that was obtained was concentrated under reduced pressure at 50°C using an evaporator. Next, the concentrated solution that was obtained was dried under reduced pressure and 9.2 g of brown crystals (extract) was obtained.

[0013] In addition, 15.6 g, 8.8 g and 11.9 g of extracts were obtained, respectively, in the same way as described above from amounts of approximately 100 g of black soybeans, achene of Siberian cocklebur and Milletia reticulata, which were the crude drugs.

[0014]

[Example 2] In this example, determinations were made of the tyrosinase activity inhibiting action of the extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata that were obtained in Example 1. Determination of the tyrosinase inhibiting activity was obtained using a method in which dopachrome [sic] produced by tyrosinase from dopa was determined quantitatively by measuring the absorbance at 475 nm. The following reaction reagents were used in the determinations of tyrosinase activity inhibiting action.

(a) Sodium succinate buffer (100 mM, pH 5.5)

(b) Mushroom tyrosinase (manufactured by Sigma Company) solution (prepared to 270 units/ml with buffer (a))

(c) L-dopa (manufactured by Wako Junyaku Co. (Ltd.)) solution (prepared to 5 mM with buffer (a))

First, 1.8 ml of buffer (a) and 0.1 ml of tyrosinase solution (b) were introduced into a test tube, 0.1 ml of a test material solution of a concentration of 2% (w/v) (aqueous solution of the extract obtained in Example 1) was added to this test tube and

the materials were incubated for 15 minutes in a constant temperature water tank at 30°C. Next, 1 ml of L-dopa solution (c) was added to the test tube and the mixture was stirred, after which said test tube was set at an inclination of approximately 45°in a reciprocating vibrator in a constant temperature chamber at 30°C and it was shaken for 40 minutes (reciprocating frequency of 150/minute). After shaking, absorbance was determined at 475 nm using a spectrophotometer and the determined value was designated as A.

[0015] As a control, the same procedure as described above was carried out except that the buffer (a) was added instead of the test material solution. Absorbance was determined at 475 nm and the determined value was designated as B. In addition, as a blank, the same procedure as described above was performed except that buffer (a) was added instead of the L-dopa solution. Absorbance was determined at 475 nm and the determined value was designated as C.

[0016] The tyrosinase activity inhibition rates of the test material solutions were calculated from the aforementioned determined values of absorbance at 475 nm. The calculations of tyrosinase activity inhibition rates were performed using the following equation. The results are shown in Table 1.

[0017] Tyrosinase activity inhibition rate (%) =
$$\{1 - (A - C)/B\} \times 100$$

[0018]

[Table 1]

Tyrosinase activity inhibitory action	
2% (w/v) aqueous solution of crude drug extract	Tyrosinase activity inhibition rate (%)
Mulberry cuttings	71
Black soybeans	72
Achene of Siberian cocklebur	69
Milletia reticulata	96

[0019] As can be seen from Table 1, extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata strongly inhibited tyrosinase activity even at the low concentration of 2% (w/v) aqueous solutions. It was confirmed that they have superior tyrosinase activity inhibiting action.

[0020]

[Example 3] In this example, determinations were made of the melanin production inhibitory action of the extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata that were obtained in Example 1.

[0021] First, B16 melanoma cells (B16-F0, ATCC No. CRL-6322), which are malignant melanoma cells that produce melanin and that originate from mice were cultured in Eagle's MEM culture medium to which bovine fetal serum had been added to give a final concentration of 10%. Amounts of 6 ml of the aforementioned culture medium containing said cells in a concentration of 3 x 10^3 cells/ml were introduced into each well of a 6-well plate (manufactured by the Falcon Company) and the materials were cultured for 5 days in a CO_2 incubator (5% CO_2, 37°C).

[0022] This culture medium was replaced with fresh Eagle's MEM culture medium (6 ml) containing 0.03% theophylline (manufactured by the Sigma Company and a suitable quantity of test material solution (aqueous solution of the extract obtained in Example 1) was added to each well, after which the materials were cultured for an additional 3 days. After culturing was completed, said culture medium was discarded, 1 ml of physiological saline solution was added to each well and the cells that were attached to the bottom faces of the wells were scraped off using a scraper and were suspended in the solution. Next, said cell suspension was transferred using a pipette to a microcentrifuge tube (1.5 ml capacity, manufactured by the Eppendorf Company) and it was centrifuged (1000 x g, 15 minutes).

[0023] As a control, a sterilized solution was added in place of the test material solution and the same experiment as described above was carried out. Further, as an experimental group for testing whitening of cells, amounts of (a) 60 μl, (b) 150 μl and (c) 300 μl of 2% aqueous solution of L-ascorbic acid were added in place of the test material solution and the same experiment as described above was carried out.

[0024] Next, the degree of whitening of cells that had been converted into pellets was compared visually and evaluations of melanin production inhibiting effect were made. The degree of whiteness of the cells in the control experimental group (group in which sterilized water was added) was taken as "-" and the degree of whiteness of the cells in the comparative experimental groups to which L-ascorbic acid had been added were taken, respectively, as (a): "+," (b): "++" and (c): "+++." The degree of whiteness of cells when test material solution was added was evaluated visually in correspondence to -, +, ++ and +++, with the strength of the melanin production inhibitory effect of the test material solution thus being evaluated in 4 levels. The results are shown in Table 2.